



TITLE:

Adult hookworms (*Necator* spp.) collected from researchers working with wild western lowland gorillas

AUTHOR(S):

Kalousová, Barbora; Hasegawa, Hideo; Petrelková, Klára J.;
Sakamaki, Tetsuya; Kooriyma, Takanori; Modrý, David

CITATION:

Kalousová, Barbora ...[et al]. Adult hookworms (*Necator* spp.) collected from researchers working with wild western lowland gorillas. *Parasites and Vectors* 2016, 9: 75.

ISSUE DATE:

2016-02-09

URL:

<http://hdl.handle.net/2433/212464>

RIGHT:

© 2016 Kalousová et al. This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (<http://creativecommons.org/licenses/by/4.0/>), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made.

RESEARCH

Open Access



Adult hookworms (*Necator* spp.) collected from researchers working with wild western lowland gorillas

Barbora Kalousová^{1*†}, Hideo Hasegawa^{2†}, Klára J. Petrželková^{3,4,5}, Tetsuya Sakamaki⁶, Takanori Kooriyama⁷ and David Modrý^{1,4,8}

Abstract

Background: In general, studies on the diversity of strongylid nematodes in endangered host species are complicated as material obtained by non-invasive sampling methods has limited value for generic and species identification. While egg morphology barely allows assignment to family, the morphology of cultivated infective third stage larvae provides a better resolution at the generic level but cannot be used for exact species identification. Morphology-based taxonomic approaches greatly depend on the examination of adult worms that are usually not available.

Methods: Hookworm parasites in two European researchers, who participated in gorilla research in the Central African Republic, were expelled after anthelmintic treatment to the faeces, collected and morphologically examined. A male worm discharged naturally from a wild bonobo (*Pan paniscus*) in Congo was also examined for comparison.

Results: Two species of *Necator* were identified in researchers' faecal material: *Necator americanus* (Stiles, 1902) and *N. gorillae* Noda & Yamada, 1964; the latter species differed in having a smaller body, smaller buccal cavity and shorter spicules with spade-shaped membranes situated distally. Males of *N. gorillae* also possessed unusual cuticular thickenings on the dorsal side of the prebursal region of the body. These characters, shared with the male worm from the bonobo, correspond well to the description of *N. gorillae* described from gorillas in Congo.

Conclusions: Based on the morphology of the hookworms recovered in this study and previous molecular analyses of larvae developed from both humans and western lowland gorillas (*Gorilla gorilla gorilla*) from this locality, we conclude that the researchers became infected with gorilla hookworms during their stay in the field. This is the first report of infection with a *Necator* species other than *N. americanus* in humans.

Keywords: *Necator* spp, *Necator gorillae*, *Necator americanus*, Hookworm, Morphology, Human infection

Background

Strongylid nematodes are an important component of helminth communities found in large herbivorous mammals [1, 2]. In general, studies on their diversity in endangered host species are complicated as material obtained by non-invasive sampling methods has limited value for generic and species identification. While egg morphology barely allows assignment to family, the morphology of cultivated infective third stage (L3) larvae provides

a better resolution at the generic level [3] but cannot be used for exact species identification. Morphology-based taxonomic approaches greatly depend on the examination of adult worms, which are mostly obtained only during necropsies and thus are lacking. As a result DNA-based taxonomy suffers from the absence of comparative sequences from well-identified individuals.

Until recently, it was believed that *Necator americanus* (Stiles, [4]) is the only species of *Necator* parasitic in humans [5]. However, Hasegawa et al. [6] recently proved by DNA sequence analysis from infective third stage larvae raised from faecal cultures that at least two *Necator* spp. are shared by humans, western lowland gorillas *Gorilla gorilla gorilla* Savage, and central

* Correspondence: barafrikacar@gmail.com

†Equal contributors

¹Department of Pathology and Parasitology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Palackého tr. 1946/1, 612 42 Brno, Czech Republic

Full list of author information is available at the end of the article



chimpanzees *Pan troglodytes troglodytes* Blumenbach, in the tropical forest in Dzanga Sangha Protected Areas (DSPA), Central African Republic (CAR). The L3 larvae sequenced showed morphological characteristics of *Necator* spp., having a distinct spear-like structure in the buccal cavity and clear transverse striations on the sheath [3]. However, it was impossible to assign the larvae to species based on their morphology. Based on the DNA sequence profile (ITS rDNA and *cox1* mtDNA), one of the detected species was considered as typical *N. americanus*, while the taxonomic placement of the second taxon was impossible and was referred to as *Necator* sp.

After returning from the field survey of gorillas in DSPA (CAR), three European researchers were diagnosed with hookworm infections. DNA sequencing on the L3 larvae cultured from two of them suggested a mixed infection with *N. americanus* and other *Necator* spp. We attempted to collect the adult hookworms from their faeces after anthelmintic treatment. Here we present the morphology of the hookworms recovered as the first report of a species of *Necator* other than *N. americanus* from humans.

Methods

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee (Ethical Commission of the Biology Centre of the Academy of Sciences of the Czech Republic) and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Two researchers studying western lowland gorillas in DSPA (CAR) were coproscopically diagnosed positive for strongylid nematodes. Both were treated by albendazole (400 mg, a single dose) and all expelled faeces during and 2 days after the treatment were collected and fixed in 4 % formaldehyde solution.

Researcher A: A 23-year-old woman who spent nine months in the field in DSPA from November 2010 to August 2011. Hookworm infection was diagnosed 11 months after returning from CAR.

Researcher B: A 37-year-old woman who has repeatedly participated in DSPA field research for 2–3 month periods in 2007–2012. She was diagnosed with hookworm infection in October 2012.

Fixed faeces were washed with running tap water on piled strainers with a mesh aperture size of 2.8 mm, 1.00 mm, 0.60 mm, 0.25 mm, and 0.106 mm, respectively. The remaining residue on each strainer was transferred to a glass dish, examined under a stereomicroscope for the presence of nematodes; the worms collected were preserved in 70 % ethanol. For light microscopy examination, the nematodes were cleared in glycerol-ethanol solution by evaporation of ethanol, and mounted on glass slides with

50 % glycerol aqueous solution, or cleared in lactophenol solution. The spicules were excised from one worm using a fine needle to observe its distal ends. All measurements are based on glycerol-mounted specimens and are presented in micrometres unless indicated otherwise. Drawings are made with the aid of a Nikon drawing tube attached to a Nikon Optiphot microscope equipped with interference contrast.

Comparative material examined: a single male adult of *Necator gorillae* from the bonobo *Pan paniscus* Schwarz from Wamba, Congo, fixed in 4 % formaldehyde solution; 20 male and 20 female *N. americanus* adult worms from a woman in Oita, Japan already identified [7].

The specimens are deposited at the Department of Pathology and Parasitology, University of Veterinary and Pharmaceutical Sciences, Brno (Czech Republic) under accession numbers VFUNK1, VFUNB1–VFUNB16.

Results

Seventeen individuals of *Necator* spp. were recovered from the researcher A and a single worm was recovered from researcher B. These were identified as two species based on detailed morphological study as described below.

Morphological descriptions

Necator americanus (Stiles, [4]) from a human

[Based on two males and nine females from researcher A and one male from researcher B.]

General. Anterior extremity strongly tilted dorsally; posterior body bent ventrally. Cuticle thick, with transverse striations. Oral aperture oval. Buccal capsule well developed. Buccal collar absent. Ventral cutting plates well developed, with slightly angular free edge corner; dorsal cutting plates present. Dorsal cone supported by two subventral plates (Fig. 1a, b); oesophagus club-shaped.

Male ($n = 3$). Body length 7.07–8.54 mm; width 344–390 at mid-body. Buccal capsule 106–114 × 89–99. Oesophagus 679–698 long, 134–154 wide near posterior end ($n = 3$). Nerve-ring 334–425 ($n = 2$), deirids and excretory pore 425–439 ($n = 2$) and 397–410, respectively, from cephalic extremity. Spicules slender, 865–975 long (corresponding to 11.6–12.9 % of worm length) with fused distal ends; one spicule forming recurved barb, the other straight, forming a spear (Fig. 2a, b). Dorsal bursal rays much shorter than laterals, diverged from each other at base, and distally bifid into unequal offshoots (Fig. 2a).

Female ($n = 9$). Body length 7.03–14.2 mm; width 299–540 at mid-body. Buccal capsule 96–123 × 83–107. Oesophagus 594–830 long, 128–154 wide near posterior end. Nerve-ring 297–585, deirids and

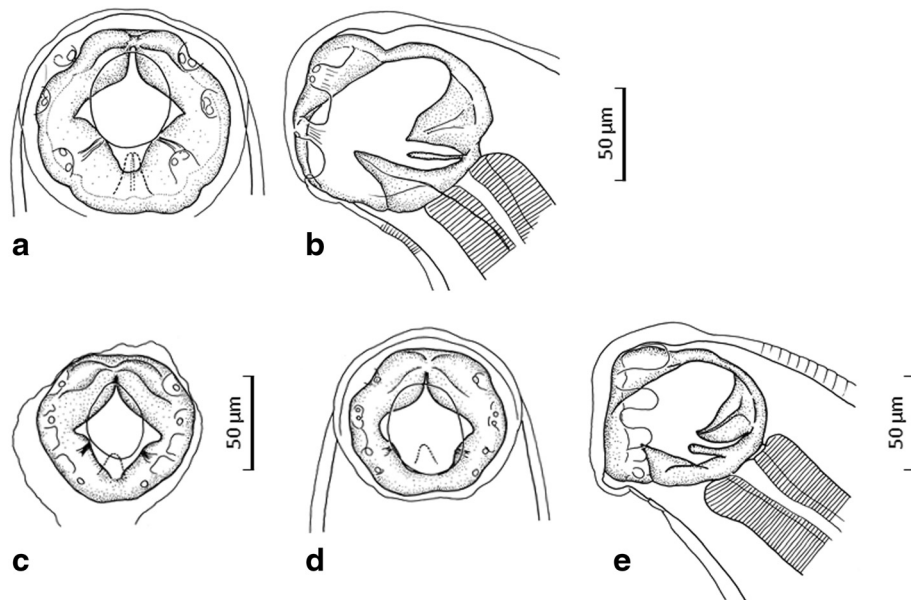


Fig. 1 Cephalic end of *Necator americanus* (a, b) and *N. gorillae* (c–e). a, b, Male of *N. americanus* collected from a researcher, dorsal (a) and right lateral (b) views; c, Male of *N. gorillae* collected from a researcher, dorsal view; d, e Male of *N. gorillae* collected from a bonobo, dorsal (d) and right lateral (e) views

excretory pore 311–618 and 247–618, respectively, from cephalic extremity. Vulva 2.40–5.93 mm from cephalic extremity (corresponding to 33.1–46.1 % of body length). Tail conical, pointed, lacking terminal spike, 182–274 long.

***Necator gorillae* Noda & Yamada, [8] from a human**

[Based on five males and two females from researcher A.].

General. Resembling *N. americanus* but smaller. Oral aperture oval. Ventral cutting plates well developed, with

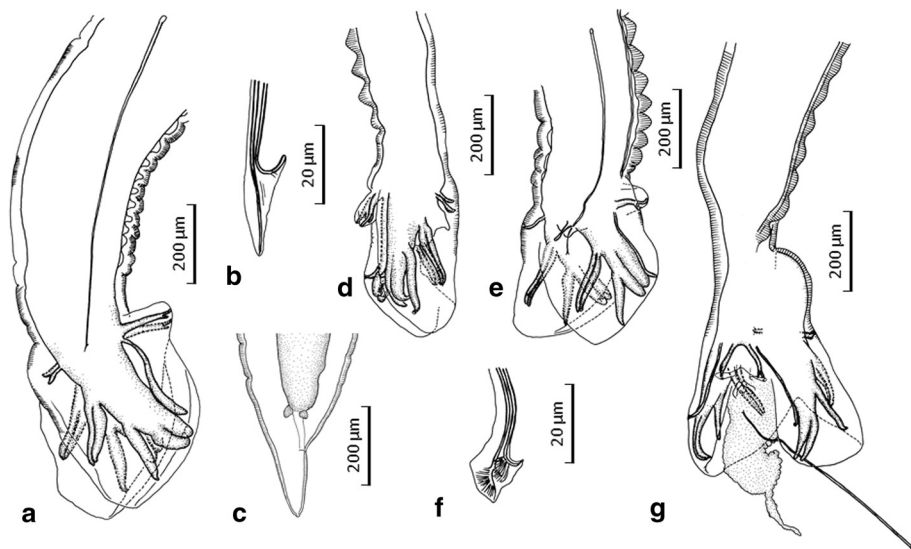


Fig. 2 Posterior extremities and distal end of spicules of the hookworms studied. a, b, Male of *N. americanus* collected from a researcher, left lateral view (a) and its spicule end (b) (note wrinkled dorsal cuticle in the prebursal region); c, Female of *N. gorillae* collected from a researcher, left lateral view; d, Male of *N. gorillae* collected from a researcher, right lateral view (note cuticular thickenings in the dorsal prebursal region); e, f, Male of *N. gorillae* collected from a researcher, left lateral view (e) (note cuticular thickenings in the dorsal prebursal region) and spicule end excised (f); g, Male of *N. gorillae* collected from a bonobo, with body twisted at base of bursa and inside matter with spicules extruded from the posterior end; note cuticular thickenings in the dorsal prebursal region

round free edge corner; dorsal cutting plates often overlapped by oral aperture rim (Fig. 1c).

Male (1 entire worm and 4 fragmented worms lacking anterior body). Body length 4.88 mm ($n = 1$); width 285–286 at mid-body. Buccal capsule 88×75 (Fig. 1c). Oesophagus 514 long, 70 wide near posterior end. Fragmented worms with 7–9 cuticular thickenings on dorsal side of prebursal portion (Fig. 2d, e); entire male with greatly wrinkled cuticle in posterior body, obscuring cuticular thickenings. Spicules slender, 489–566 long (corresponding to 10.1 % of worm length) ($n = 1$); one spicule recurved, the other straightened distally; small spade-shaped membrane with fine lines connecting both distal ends of spicules present (Fig. 2d, e). Bursal rays thin except lateral rays; ventral rays running together along whole length; externolateral rays diverged from mediolateral rays, widely separated distally; externodorsal rays very thin, attached to posterolateral rays along nearly entire length, but distally diverged; dorsal rays thin, diverged from each other at base, and divided distally into two unequal offshoots (Fig. 2d, e).

Female ($n = 2$). Body length 6.00–6.19 mm; width 293–390 at mid-body. Buccal capsule $98\text{--}99 \times 84\text{--}88$. Oesophagus 580–585 long, 138–141 wide near posterior end. Nerve-ring, deirids and excretory pore 288–354, 170–354 and 189–278, respectively, from cephalic extremity. Vulva 2.24 mm from cephalic extremity (corresponding to 37.3 % of body length) ($n = 1$). Tail conical, pointed, lacking terminal spike, 169–173 long (Fig. 2c) (morphology identical with that of *N. americanus*).

***Necator gorillae* Noda & Yamada, [8] from a bonobo**

Male ($n = 1$). Morphology identical with *N. gorillae* from humans (see above). Body length 7.38 mm, width 312 at mid-body. Buccal capsule 88×77 (Fig. 1d, e). Oesophagus 598 long, 122 wide. Nerve-ring, deirids and excretory pore 321, 481 and 411, respectively, from cephalic extremity. Spicules 570 long (corresponding to 7.7 % of worm length). Seven transverse cuticular thickenings present on dorsal side of prebursal portion (Fig. 2g).

Remarks

The worms identified as *N. americanus* in the present study were morphologically identical to *N. americanus* collected from a woman in Japan, including the cephalic structure and distal ends of the spicules [9, 10]. The presence of *N. americanus* in the same material from researcher B was previously proved by sequence analysis of DNA from L3 larvae raised by coproculture [6].

We compared the specimens of *N. gorillae* identified in our study with the species previously described in great apes [11, 12], i.e. *N. exilidens* Cummins, [13], *N.*

congolensis Geddoelst, [12] and *N. gorillae* Noda & Yamada, [8]. *Necator exilidens* differs from the other two species in the shape of the mouth, the length of the spicules and the shape of the ventral cutting plates. *Necator exilidens* has spindle-shaped mouth whereas the mouth in the other two species has an ovoidal shape. The spicules of *N. exilidens* are also more than twice as long (1,360 μm) in comparison with those of *N. congolensis* and *N. gorillae* (both $< 600 \mu\text{m}$). All male specimens of *N. gorillae* recovered in our study possess spicules shorter than 600 μm with distal small spade-shaped membrane with fine lines. Ventral cutting plates of *N. exilidens* are round (*vs* angular in the other species). Because of all of these characteristics, the distinction of the present material from *N. exilidens* is apparent. The difference between *N. congolensis* and *N. gorillae* comprises the absence of dorsal cutting plates in *N. congolensis*, which are present in *N. gorillae* examined here. The most important aspect which distinguishes *N. gorillae* from other *Necator* spp. is the presence of prebursal dorsal cuticular thickenings; these ridges do not seem to be resulting from body constriction as the subcuticle layer showed no wrinkles. This is in sharp contrast with the almost smooth dorsal cuticle in *N. americanus* (Fig. 2a) and other previously described species [12]. The prebursal dorsal thickenings have been described only for *N. gorillae* collected from a wild western lowland gorilla, which was caught in Congo, transported to Japan and died soon after arrival [8]. Additionally, the male hookworm from the comparative bonobo material shared morphological characteristics with *N. gorillae* (Figs. 1a, 2g) and we assume these to be conspecific. Other morphological features and measurements of the male *N. gorillae* from the researcher and the bonobo also agree well with those of *N. gorillae*.

In the original description of *N. gorillae* by Noda & Yamada [8], the buccal capsule was given as $66\text{--}68 \times 58\text{--}60 \mu\text{m}$ in males and $68\text{--}74 \times 60\text{--}66$ in females, i.e. much smaller than in the present worms identified as *N. gorillae*. However, the buccal cavity of the male shown in Fig. 2 in Noda & Yamada [8] measures $88 \times 80 \mu\text{m}$, i.e. almost equal to that in males of *N. gorillae* described here from humans and the bonobo. Finally, the morphology of the spicules of *N. gorillae*, as described by Noda & Yamada [8], corresponds to that observed by us in the worms identified as *N. gorillae*, regardless of some terminological inconsistencies.

Discussion

The species composition of *Necator* spp. parasitising African great apes is complex and remains unclear. Four species, i.e. *Necator americanus*, *N. exilidens* (from a chimpanzee), *N. congolensis* (from chimpanzees in Congo) and *N. gorillae* (from western lowland gorilla in

Congo), have been described [4, 8, 12, 13]. The key distinguishing features of the above-mentioned species are not clear, especially those of *N. exilidens*, *N. congolensis* and *N. gorillae*. As the type-material of these species is not available at the present time, direct comparison with our specimens was impossible. Nevertheless, the original description of *N. gorillae* outlined by Noda & Yamada [8] is detailed enough to allow thorough morphological comparison as presented above.

This study is a follow up to previous work by Hasegawa et al. [6], who described the DNA sequence profile (ITS and *cox1*) of L3 larvae raised from stools of researchers and confirmed that researchers (and great apes) at DSPA were infected with more than one species of *Necator*. Initially identified *Necator* spp. clustered in both rDNA and mtDNA trees with *N. americanus*, the most common human-infecting species of *Necator*; this corresponds well to the presence of adult *N. americanus* described in our study. The present specimens of *N. americanus* exhibited identical morphology with those expelled from the Japanese woman [7]. It is also noteworthy that *Necator* sp. with ITS2 sequence closely resembling the type III previously recorded from a human in DSPA [6], was recently found in bonobo from the Congo [14].

Other genotypes/haplotype groups, dominant among larvae obtained from gorillas and chimpanzees, but present also in humans, were suggested to belong to other previously described great ape hookworm taxa i.e., *N. congolensis*, *N. exilidens* or *N. gorillae* [6]. Based on the morphological data obtained and presented in this manuscript, the *Necator* specimens, different from *N. americanus* and shared by researchers and gorillas at DSPA, are identified as *N. gorillae*. The possible synonymy of *N. gorillae* with *N. congolensis* (and possibly also with *N. exilidens*) cannot be ruled out, regardless of the fact, that the two latter species were described from chimpanzees. Interestingly, at the time of the original description of *N. congolensis* by Geddoelst [12], the bonobo has not yet been described as a distinct species. It is probable, that at least one of the two “chimpanzees” that were the source of the type-material of *N. congolensis* originated from Busira Region in Congo, which is, in fact, an area inhabited by bonobos.

Nematode identification has traditionally relied on morphometric data and comparison of morphological structures; morphology-based diagnostics of the nominal species should be further extended by molecular data [15]. Taxonomy is currently dealing with an explosion of sequence, genomic, proteomic and other molecular data [16]. However, the fact that the genomic sequences deposited in databases (EMBL, GenBank, etc.) sometimes refer to misidentified or unidentified organisms complicates analyses [17]. Each DNA sequence should ideally

be accompanied by comprehensive identification of the specimen [17–19]. However, this is difficult to achieve with no clear taxonomic check on the name given to a sequence and usually no reference material is retained [17]. Moreover, sequences from the majority of nominal nematode taxa are missing [15]. Generally, the holotype which is declared as the ‘name-bearing’ specimen is too valuable to be ground-up for DNA isolation. Furthermore, type-specimens have often been preserved (sometimes for centuries) with fixatives prejudicial to the preservation of nucleic acid. As such, frequently, the types are absent.

The example of *Necator* spp. in primates and humans shows two possible options in resolving this dilemma. We can give up traditional taxonomy and rely on OTUs based on derived sequences. This approach is broadly used for avian malaria parasites [20]. Alternatively, a maximum effort should be made to collect new well-preserved material, which will be identified by experts and subsequently sequenced. The second challenging option is the only way to continue using the traditional taxonomy important for parasites such as hookworms. This will largely depend on the collaboration between laboratories, institutions, field researchers and wildlife veterinarians studying great apes and their parasites.

Conclusions

DNA sequences of L3 larvae showed plural species of *Necator* in both humans and great apes in the Central African Republic previously. Based on morphological analyses, we identified the adult hookworms recovered from the faeces of two researchers, in which larvae were also included and identified by Hasegawa et al. [6], as *N. gorillae* and *N. americanus*. This is the first report, supported by the morphology of adult worms, of a species of *Necator* other than *N. americanus* in humans. In order to maintain traditional nematode diagnostics we urge collection of new well-preserved adults suitable for both morphological and molecular examination.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Collection of the material: BK, KJP, TS, TK. Processing the material before description: HH, BK. Morphological description: HH. Wrote the draft of the manuscript: BK, HH, DM. Revised the manuscript: BK, DM, KJP. All authors read and approved the final version of the manuscript.

Acknowledgements

This publication is an outcome of the HPI-lab (Laboratory for Infectious Diseases Common to Human and Non-Human Primates) co-financed from European Social Fund and state budget of the Czech Republic (project OPVK CZ.1.07/2.3.00/20.0300). We thank the following granting agencies for their generous support of this research. Research was supported by the Grant Agency of the Czech Republic (15-05180S), by the project CEITEC-Central European Institute of Technology (CZ.1.05/1.100/02.0068) from European Regional Development Fund, Internal Grant Agency of University of Veterinary and Pharmaceutical Sciences Brno (90/2014/FVL), the institutional support of the Institute of

Vertebrate Biology Academy of Sciences of the Czech Republic (RVO: 68081766) and the Global Environmental Research Fund (F-061) of the Japanese Ministry of Environment. We would like to thank the government of the Central African Republic and the World Wildlife Fund for granting permission to conduct our research in the Central African Republic; the Ministre de l'Education Nationale, de l'Alphabetisation, de l'Enseignement Supérieur, et de la Recherche for providing research permits; and the Primate Habituation Programme, especially Angelique Todd for providing logistical support in the field.

Author details

¹Department of Pathology and Parasitology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences, Palackého tr. 1946/1, 612 42 Brno, Czech Republic. ²Department of Biology, Faculty of Medicine, Oita University, Hasama, Yufu, Oita 879-5593, Japan. ³Institute of Vertebrate Biology, Academy of Sciences of the Czech Republic, Kvetná 8, 603 65 Brno, Czech Republic. ⁴Biology Centre, Institute of Parasitology, Academy of Sciences of the Czech Republic, Branisovská 31, 370 05 České Budějovice, Czech Republic. ⁵Liberec Zoo, Lidové sady 425/1, 460 01 Liberec, Czech Republic. ⁶Primate Research Institute, Kyoto University, Inuyama, Aichi 484-8506, Japan. ⁷Department of Veterinary Science, School of Veterinary Medicine, Rakuno Gakuen University, 582 Bunkyo-dai-Midori, Ebetsu, Hokkaido 069-8501, Japan. ⁸Central European Institute for Technology (CEITEC), University of Veterinary and Pharmaceutical Sciences, Palackého 1946/1, 612 42 Brno, Czech Republic.

Received: 2 October 2015 Accepted: 2 February 2016

Published online: 09 February 2016

References

- Arneberg P, Skorping A, Grenfell B, Read AF. Host densities as determinants of abundance in parasite communities. *Proc R Soc Lond B Biol.* 1998;265:1283–9.
- Arneberg P. Host population density and body mass as determinants of species richness in parasite communities: comparative analyses of directly transmitted nematodes of mammals. *Ecography.* 2002;25:88–94.
- Little MD. Differentiation of nematode larvae in coprocultures: Guidelines for routine practice in medical laboratories. WHO Tech Rep Ser. 1981;666:144–50.
- Stiles CW. A new species of hookworm (*Uncinaria americana*) parasitic in man. *Am Med.* 1902;3:777–8.
- Tang YT, Gao X, Rosa BA, Abubucker S, Hallsworth-Pepin K, Martin J, et al. Genome of the human hookworm *Necator americanus*. *Nat Genet.* 2014;46:261–9.
- Hasegawa H, Modrý D, Kitagawa M, Shutt KA, Todd A, Kalousová B, Profousová I, Petrželková KJ. Humans and great apes cohabiting the forest ecosystem in Central African Republic harbour the same hookworms. *PLoS Negl Trop Dis.* 2014;doi:10.1371/journal.pntd.0002715.
- Inoue K, Ozaka S, Okamoto K, Ogawa R, Mizukami K, Okimoto T, et al. Multiple infections with helminths – whipworm, hookworm, and roundworm. *Endoscopy.* 2014;46:117–8.
- Noda R, Yamada H. On two species of nematodes, *Necator gorillae* sp. nov. (Ancylostomidae) and *Chitwoodspirura wehri* Chabaud and Rousselot, 1956 (Spiruridae), from a gorilla. *Bull Univ Osaka Pref Ser B.* 1964;15:175–80.
- Looss A. The anatomy and life history of *Agchylostoma duodenale* Dub. A monograph. Part II: The development in the free state. *Rec Sch Med.* 1911;4:159–613.
- Ackert JE, Payne FK. Investigations on the control of hookworm disease. XII. Studies on the occurrence, distribution and morphology of *Necator suillus*, including descriptions of the other species of *Necator*. *Am J Hyg.* 1922;3:1–25.
- von Linstow OFB. The American hookworm in chimpanzees. *Am Med.* 1903;6:611.
- Gedoelst L. Notes sur la faune parasitaire du Congo belge. *Rev Zool Afr.* 1916;5:1–90.
- Cummins SL. The anatomy and life history of *Agchylostoma duodenale* (Dubini) by Prof. A. Looss. *J Roy Army Med Corps.* 1912;19:42–55.
- Narat V, Guillot J, Pennec F, Lafosse S, Grüner AC, Simmen B, Ngawolo JCB, Krief S. Intestinal helminths of wild bonobos in forest-savanna mosaic: Risk assessment of cross-species transmission with local people in the Democratic Republic of the Congo. *EcoHealth.* 2015; doi:10.1007/s10393-015-1058-8.
- Powers T. Nematode molecular diagnostics: From bands to barcodes. *Annu Rev Phytopathol.* 2004;42:367–83.
- Charles H, Godfray J. Challenges for taxonomy. *Nature.* 2002;417:17–9.

- Stevens JR, Schofield CJ. Phylogenetics and sequence analysis – some problems for the unwary. *Trends Parasitol.* 2003;19:582–8.
- Blaxter ML. The promise of a DNA taxonomy. *Philos Trans R Soc Lond B Biol Sci.* 2004;359:669–79.
- Ratnasingham S, Hebert PDN. BOLD: The Barcode of Life Data System (www.barcodinglife.org). *Mol Ecol Notes.* 2007;7:355–64.
- Bensch S, Hellgren O, Pérez-Tris J. MalAvi: a public database of malaria parasites and related haemosporidians in avian hosts based on mitochondrial cytochrome b lineages. *Mol Ecol Resour.* 2009;9:1353–8.

Submit your next manuscript to BioMed Central and we will help you at every step:

- We accept pre-submission inquiries
- Our selector tool helps you to find the most relevant journal
- We provide round the clock customer support
- Convenient online submission
- Thorough peer review
- Inclusion in PubMed and all major indexing services
- Maximum visibility for your research

Submit your manuscript at
www.biomedcentral.com/submit

